

Exhibit 3

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2/2 Digest FT7 DNA 3, 10, 14 w/ Kpn I / Hind III
use previous prep as control (-)

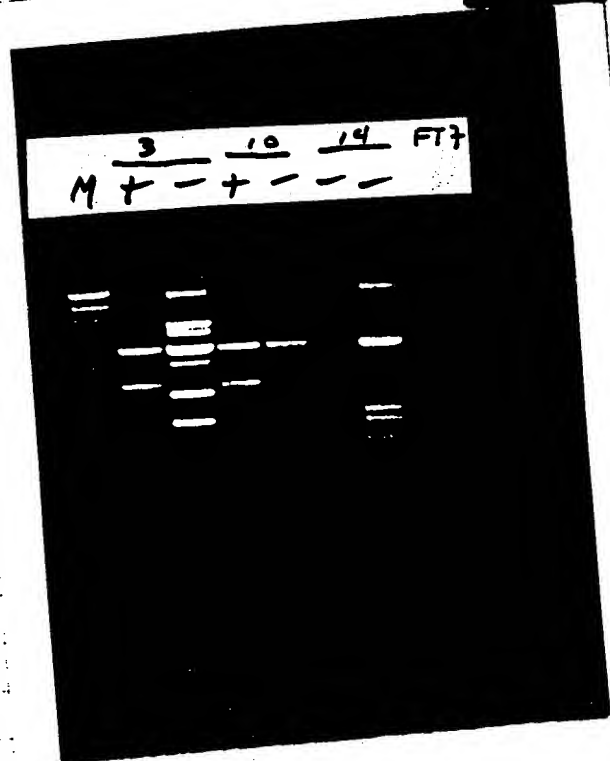
Expected Size

(3) Sense (4) Antis. (-)

3159	3159
1573	1366
625	782

10	3159	3159
	1573	1124
	650	832
	380	650

14	3159	3159
	1573	945
	650	832
	204	650



Maybe problem w/ #14

Some how samples got mixed-up

60 band is checked out - Sheet for single colonies

A B : E

Also mini prep from original culture

Digest w/ Kpn I / Hind III 0/14

4/23 Run gel of Digests

Clearly Sample A which was given as mate prep is in the wrong orientation

Start 0/14 of D : E to mini prep before start of 500ml culture

4/24 mini prep FT7 DNA 14 - D : E

Digest w/ Kpn I / Hind III

run on gel - Both are fine
use -D for mate



M D E

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4th Plasmid isolation of FT7, cDNA 14 - D up to Binding
 - do 500 nls in 2 250 sets, extract 2 tubes to Band
 7th Pull Bands - double band 1 pip - 6 hrs during the Day

7th Digest FT7, cDNA 14 w/ Kpn I / Hma III

- ① - Single bandy
- ② Double Bandy
- ③ FT7, cDNA 14 antisense



Check Absorbance / Concentration of

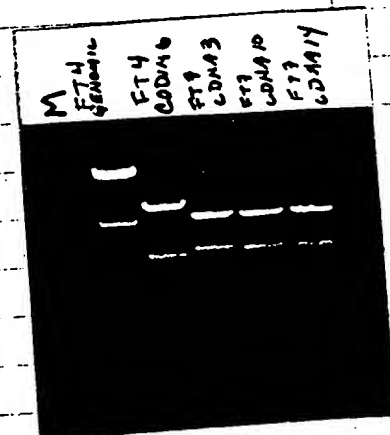
Samples	260	FT7	cDNA 14	260/280
①	.127	.062	.63 ng/ul	2.0
②	.143	.065	.71 ng/ul	2.3

Sequence
 FT7, cDNA 3
 T7

cDNA 14
 T7
 8850
 9007
 8874

Digest Mouse FT4

	260	280	260/280
psr7 genomic	.085	.045	
psr7 Coding	.106	.060	
Digest of cDNA 3			
Kpn I cDNA 10			
Hma III cDNA 14			



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8/4 FACS Analysis of Transfected cells w/ following Vectors

pCDNA 7

FIT7, 1, 2a, 2b, 3

1, 2b, 3

1, 3

cDNA 3

cDNA 10

cDNA 14

2 plates / Vector - Divide

FACS 7mL

yuko 5mL

EAT 3mL

ETassy 5mL

(75ul)

(100ul)

Antibodies

IgM H - blank 1:100

IgM hrf green 1:1000

IgM SLx 1:200

IgG ha red 1:500

IgG SLa blue 1:500

2nd Antibody

IgM 2.5mL

12.5 / 2.5mL

IgG 1.5

6.0 / 1.5mL

Results are

H - all neg

hrf - all neg

SLx, pCD (-), 1, 2a, 2b, 3 (+), 1, 2b, 3 (+), 1, 3 (-), cDNA 3 (-), 10 (+), 14 (+)

ha all neg

SLa all neg

9/2 Spin 12 punn KG, FT4

6451

6080

2470

6199

6374

6087

6306

6086

6203

6085

5721

5671

9/9 Run sy gel of above samples

Also spin

5728

6084

7213

5731

5737

6082

5662

5727

6201

5725

6200

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8/10 Spinning gel of 8/9 samples
Sequence

FT4 6079

6202

6307

6373

CDNA10 715

946

CDNA14

T7

8850

8807

8874

Protein assay of FACS Samples, Also CAT assay

pcDNA

FT7 1,2a2b,3

FT7 1,2b,3

FT7 1,3

CDNA3

CDNA10

CDNA14

BSA Blank Protein

0 1.00 116 108

1 0.208 225 214

2 0.369 383 376

4 0.691 673 682

8 1.215 1.230 1.222

16

Sample

pcDNA .292 .294 .293

1,2a2b,3 .337 .330 .33

1,2b,3 .298 .343 .32

1,3 .369 .372 .37

CDNA3 .363 .379 .37

CDNA10 .306 .298 .3

CDNA14 .225 .253 .2

FACS Results

Only stain w/ 5h4

1,2a2b,3 23.6%

1,2b,3 24.6%

CDNA10 14.9%

CDNA14 8.0%

Micro BCA Protein Assay

Reagent mbc	MC	MB	MA
Per assay tube (ml)	0.01	0.24	0.25
Cocktail for	Tubes		

Incubate 1 h at 60°C and cool to room temp.

Since the color development has no end point, all tubes must be heated and cooled at the same time

1 mg/ml BSA (l)	Water (l)	Reagent (l)	Abs. 562	
0.0	500.0	500.0	Blank	
1.0	499.0	500.0	0.108	Slope = 0.0734
2.0	498.0	500.0	0.214	Y intercept = 0.0656
4.0	496.0	500.0	0.376	X intercept = -0.8940
8.0	492.0	500.0	0.682	R = 0.9985
16.0	484.0	500.0	1.222	

8/10 Spinning of FT4 9/10 Samples

Sample	l in assay	Water (l)	Reagent (l)	Abs. 562	mg protein/ml
pcDNA1	5.00	495.00	500	0.293	0.62
FT7 1,2a2b,3	5.00	495.00	500	0.333	0.73
FT7 1,2b,3	5.00	495.00	500	0.320	0.69
FT7 1,3	5.00	495.00	500	0.370	0.83
CDNA 3	5.00	495.00	500	0.371	0.83
CDNA 10	5.00	495.00	500	0.302	0.64
CDNA 14	5.00	495.00	500	0.239	0.47

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9. CAT Assay (FT7) samples

2.5 μ l of cell extract

Control

pcDNA

1,2a2b,3

1,2b,3

1,3

cdNA 3

cdNA 10

cdNA 14

Cocktail 15

3/4 Chlo 300

Tris, 2M pH 8.0 75

But Co A 75

H₂O 300

50/ μ l tube

CAT

Vector	Counts/5ul 8/12/94	Incorporated Counts (.95)	Total Counts	Total Counts Incorporated
pcDNA1	11,349	11,829	9,189	8,083
FT7 1,2a2b,3	11,161	11,441	27,211	21,919
FT7 1,2b,3	11,772	11,826	37,541	40,684
FT7 1,3	11,215	11,690	23,076	28,706
cdNA 3	11,834	11,206	33,885	39,098
cdNA 10	12,017	11,312	30,066	33,165
cdNA 14	11,079	11,570	44,133	40,529
Control		10,354		424
	Protein Conc. (ug/2.5ul)	Total Counts Inc- Bkg	% INC/hr	% INC/hr/ug
pcDNA1	1.55	9,249	8,083	3.92
FT7 1,2a2b,3	1.83	28,219	22,649	11.27
FT7 1,2b,3	1.72	39,093	42,401	14.32
FT7 1,3	2.07	23,867	29,793	9.65
cdNA 3	2.07	35,244	40,730	13.03
cdNA 10	1.60	31,224	34,487	11.55
cdNA 14	1.17	46,032	42,238	17.32

9. Assemble Data of FT7 FACS, CAT Assay / give to Judy
Work on FT4 Sequencer

9.5 7 deaza sequencing on Trumble's FT4 samples

6451 6200
6378 6079
6306 6307
6203 1897
5721 1898
1899

9.6 Sequencing gel of 8/15 samples (FT7) Formamide gel
Prase Brown's rather than But w/ GAP probe
To check condition of RNA

9.7 The 7 deaza technique didn't resolve all of the compressions
Try a Terminal transferase technique
Run standard Syntexase rxn, after extension made
Heat tubes (A, C, G, T) for 1.5 mins 100°C
Hold on ice 10 min, Purpore TdT/dNTP cocktail
Add to tubes, 37°C 30 min
Add Spl Stop